

LISTING OF THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method for at least one of identifying ~~and/or~~ and detecting T-cell epitopes of a protein antigen in vitro, where a population of peptide fragments of the antigen is subjected to competitive binding to a first immobilized receptor unit, preferably in the presence of a second receptor unit which, together with the first receptor unit, is capable of forming a receptor, where at least one peptide fragment with affinity to the receptor binds to at least the first, ~~preferably both,~~ receptor unit(s), and the bound peptide fragment is then isolated and analyzed, said method comprising
 - a) ~~immobilization of~~ immobilizing at least the first receptor unit which has at least one first functional group on a nanoparticle, the surface of which has at least one second functional group which binds the first functional group,
 - b) ~~preparation~~ preparing of a population of peptide fragments of the protein antigen which comprises different sequence ranges of the protein antigen,
 - c) carrying out a competitive binding of the peptide fragment population to the first receptor unit immobilized on the nanoparticle, ~~preferably in the presence of a second receptor unit,~~ where at least one peptide fragment having affinity to at least the first receptor unit, ~~preferably to both receptor units, if appropriate together with the second receptor unit,~~ binds to the first receptor unit, giving a receptor/peptide fragment complex immobilized on the nanoparticle, and

- d) ~~analysis of~~ analyzing the at least one of immobilized receptor/peptide fragment complex ~~and/or~~ and the bound peptide fragment.

Claims 2-5 (Canceled)

6. (Currently Amended) The method as claimed in ~~any of claims~~ claim 1 to 5, where the receptor is a major histocompatibility complex (MHC) molecule, the receptor/peptide fragment complex is a peptide-presenting MHC molecule and the first and the second receptor unit are chains of the MHC molecule.

Claims 7-16 (Canceled)

17. (Currently Amended) The method as claimed in ~~any of claims~~ claim 1 to 16, where the first and the second receptor unit are either natural chains or chains prepared by genetic engineering or chemical synthesis.

Claims 18-24 (Canceled)

25. (Currently Amended) The method as claimed in ~~any of claims~~ claim 1 to 24, where the population of peptide fragments of the protein antigen is prepared by a method selected from the group consisting of enzymatic protein cleavage, genetic engineering ~~or~~ and chemical synthesis.

Claims 26-34 (Canceled)

35. (Currently Amended) The method as claimed in ~~any of claims~~ claim 1 to 34, where the immobilization of the first receptor unit or the immobilization of the first and second receptor unit on the nanoparticles is carried out by incubating the receptor unit(s) with the nanoparticles in a PBS buffer for a period of 1 h to 4 h, ~~preferably 2 h~~, at room temperature in a shaking apparatus, affording nanoparticles having immobilized first receptor units or nanoparticles having immobilized first and second receptor units.

36. (Currently Amended) The method as claimed in ~~any of claims~~ claim 1 to 34, where the immobilization of receptor unit(s) on the nanoparticles is carried out by preparing in solution a receptor/peptide complex using a peptide of known sequence and suitable length, the first receptor unit and the second receptor unit, immobilizing the receptor/peptide complex on the nanoparticles, subjecting the nanoparticles having the immobilized receptor/peptide complex to a treatment to remove at least the bound peptide, giving resulting in nanoparticles having immobilized receptor units.

Claims 37-46 (Canceled)

47. (Currently Amended) The method as claimed in ~~any of claims~~ claim 1 to 46, where the suspension of the nanoparticles having the immobilized receptor/peptide fragment complex with the bound peptide fragment is analyzed using a matrix-assisted laser desorption/ionization (MALDI) ~~methods~~ method, ~~in particular the MALDI-TOF (time-of-flight) method.~~

Claims 48-49 (Canceled)

50. (Currently Amended) The method as claimed in ~~any of claims~~ claim 1 to 46, where

the peptide fragment bound in the immobilized receptor/peptide fragment complex is removed from the complex by dissolution, isolated and analyzed.

Claims 51-54 (Canceled)

55. (Currently Amended) A method for at least one of identifying ~~and/or~~ and preparing a peptide vaccine against a protein antigen, where ~~the~~ an amino acid sequence of a T-cell epitope of the protein antigen is identified in vitro, a peptide having the identified amino acid sequence is prepared and a peptide-presenting major histocompatibility complex (MHC) is prepared using the prepared peptide and a first and second chain, which method comprises:

- a) providing a population of peptide fragments of the protein antigen,
- b) providing nanoparticles having, at their surface, at least one first immobilized chain of an MHC molecule, where the chain has a conformation which allows formation of an MHC molecule,
- c) carrying out competitive binding of the peptide fragment population to ~~the~~ a first chain immobilized on the nanoparticles in the presence of a second chain of an MHC molecule, where ~~the~~ a peptide fragment having the greatest affinity to the two chains of the MHC molecule binds together with the second chain to the first chain, giving a peptide fragment-presenting MHC molecule, and
- d) ~~isolation of~~ isolating the peptide fragment from the MHC molecule to identify a peptide fragment suitable for a peptide vaccine, and ~~determination of~~ determining its amino acid sequence, ~~and optional practice of steps e) to h), namely,~~

- ~~e) preparation, by genetic engineering or chemical synthesis, of suitable amounts of a peptide based on the determined amino acid sequence of the peptide fragment;~~
- ~~f) preparation, by genetic engineering or chemical synthesis, of suitable amounts of the first and second chains;~~
- ~~g) preparation of suitable amounts of peptide-presenting MHC molecules by joint incubation of the first chain, the second chain and the peptide prepared, and~~
- ~~h) preparation of a peptide vaccine in the form of a lyophilizate or an aqueous colloidal solution or suspension of the peptide-presenting MHC molecules.~~

Claims 56-74 (Canceled)

75. (Currently Amended) The method as claimed in ~~any of claims~~ claim 55 to 74, where the nanoparticles which have a first immobilized chain on their surface are obtained by the following steps:

- a) ~~incubation of~~ incubating the first chain which contains the first functional group, of the second chain and of a peptide whose amino acid sequence is known and which is known to be capable of forming a peptide-presenting MHC molecule under suitable conditions,
- b) ~~incubation of~~ incubating of the peptide-presenting MHC molecule with nanoparticles whose surface has at least one second functional group which binds the first functional group, under conditions suitable for immobilizing the peptide-presenting MHC molecule on the nanoparticles,
- c) ~~treatment of~~ treating the nanoparticles having the immobilized peptide-presenting

- MHC molecules with a suitable buffer to remove the second chain and the peptide having a known amino acid sequence from the immobilized MHC molecule, and
- d) ~~purification~~ purifying of the nanoparticles having the first immobilized chain.

Claims 76-81 (Canceled)

82. (Original) A method for controlling the quality of receptor/ligand complexes and/or components thereof, which comprises preparing or providing a receptor/ligand complex in solution of two receptor units, where at least one receptor unit has a first functional group, and a ligand, immobilizing the receptor/ligand complexes on nanoparticles which have, on their surface, at least one second functional group which binds the first functional group, and analyzing the nanoparticles having the immobilized receptor/ligand complex using a MALDI method.

Claims 83-88 (Canceled)

89. (Original) A method for preparing nanoparticles having, on their surface, at least one immobilized receptor unit or one immobilized receptor, which comprises
- a) preparing a receptor/ligand complex by incubation of a first receptor unit having a first functional group, a second receptor unit capable of forming, with the first receptor unit, a receptor, and a ligand in solution,
 - b) immobilizing the receptor/ligand complex formed on nanoparticles having, on the surface, at least one second functional group which binds the first functional group, and
 - c) treating the nanoparticles having the immobilized receptor/ligand complex with an

acidic buffer to release at least the bound ligand, giving nanoparticles having immobilized receptor units.

Claims 90-94 (Canceled)

95. (Currently Amended) The method as claimed in ~~claims~~ claim 89 to 94, where the receptor is an MHC molecule, the ligand is a peptide of known sequence and defined length which binds to the receptor and the receptor/ligand complex is a peptide-presenting MHC molecule.

Claims 96-104 (Canceled)

105. (Currently Amended) A method for preparing nanoparticles having immobilized peptide-presenting MHC molecules, where nanoparticles having at least one first immobilized chain of an MHC molecule ~~preparable~~ prepared by a method according to ~~any of claims~~ claim 89 to 104 are incubated in the presence of a second chain capable of forming an MHC molecule with the first chain, with a peptide capable of binding to the MHC molecule, giving a peptide-presenting MHC molecule immobilized on the nanoparticles.

Claims 106-107 (Canceled)

108. (Currently Amended) A method for at least one of enriching ~~and/or~~ and isolating specific CD4⁺-T-lymphocytes or CD8⁺-T-lymphocytes from peripheral blood mononuclear cells (PBMCs), which comprises

- a) preparing nanoparticles having immobilized peptide-presenting MHC molecules as claimed in ~~any of claims~~ claim 105 to 107, where the peptide is a T-cell epitope,
- b) isolating peripheral blood mononuclear cells from a suitable starting material,
- c) incubating the isolated blood mononuclear cells with the nanoparticles having the immobilized peptide-presenting MHC molecules, the T-lymphocytes binding to the T-cell epitope of the immobilized peptide-presenting MHC molecules, and
- d) removing the nanoparticles having the T-lymphocytes bound to the immobilized peptide-presenting MHC molecules from the unbound peripheral mononuclear cells.

Claims 109-113 (Canceled)

114. (Currently Amended) A method for at least one of priming ~~and/or~~ and restimulating a CD4⁺-T- or CD8⁺-T-lymphocyte reaction in vitro, which comprises

- a) identifying a T-cell epitope as claimed in ~~any of claims~~ claim 1 to 54 and determining its amino acid sequence,
- b) preparing a nucleic acid coding for a peptide having the amino acid sequence of the T-cell epitope,
- c) introducing the nucleic acid prepared ~~under b)~~ in step (b) into a suitable vector,
- d) introducing the vector obtained ~~under c)~~ in step (c) into dendritic cells isolated, if appropriate, from cultivated peripheral blood mononuclear cells,
- e) propagating the dendritic cells obtained ~~under d)~~ in step (d), which have the vector, in vitro, and
- f) stimulating at least one of autologous CD4⁺- ~~and/or~~ and CD8⁺-cells in vitro using the dendritic cells obtained ~~under d) or c)~~ in step (d) or (e).

115. (Currently Amended) A nanoparticle, comprising on the surface at least one receptor unit, ~~in particular an immobilized chain of an MHC molecule.~~

Claims 116-121 (Canceled)

122. (Original) A nanoparticle having an immobilized MHC molecule, where the MHC molecule comprises a first and a second chain and the MHC molecule is immobilized on the nanoparticle surface by binding of a first functional group present in the first chain to a second functional group present on the nanoparticle surface or by binding of the first functional group present in the first chain to the second functional group present on the nanoparticle surface and binding of a third functional group present in the second chain to a fourth functional group present on the nanoparticle surface.

123. (Original) A nanoparticle having a peptide-presenting MHC molecule immobilized on the nanoparticle surface, where the peptide-presenting MHC molecule comprises a first chain, a second chain and a peptide of 8 to 24 amino acids and the MHC molecule is immobilized on the nanoparticle surface by binding of a first functional group present in the first chain to a second functional group present on the nanoparticle surface or by binding of the first functional group present in the first chain to the second functional group present on the nanoparticle surface and binding of a third functional group present in the second chain to a fourth functional group present on the nanoparticle surface.

Claims 124-127 (Canceled)

128. (Currently Amended) A peptide vaccine which comprises at least one peptide-presenting MHC molecule preparable as claimed in ~~any of claims~~ claim 55 to 81 and/or which comprises at least one protein antigen which contains a T-cell epitope identifiable by the ~~methods~~ method as claimed in ~~claims claim 1 to 54~~.

Claims 129-131 (Canceled)

132. (Currently Amended) A kit for at least one of identifying ~~and/or~~ and detecting T-cell epitopes of a protein antigen in vitro, comprising a container with a suspension of nanoparticles having an immobilized MHC molecule as claimed in ~~any of claims~~ claim 122 to 127 or a container with a suspension of nanoparticles having an immobilized first chain of an MHC molecule as claimed in ~~any of claims~~ claim 115 to 121 and a container with a lyophilizate of a second chain.

133. (Currently Amended)) ~~The use of a nanoparticle as claimed in any of claims 115 to 127~~
A method for at least one of identifying ~~and/or~~ and detecting T-cell epitopes of a protein antigen in vitro wherein a nanoparticle as claimed in claim 115 is used.

134. (Currently Amended) ~~The use of a nanoparticle as claimed in any of claims 115 to 127~~
A method for preparing a peptide vaccine wherein a nanoparticle as claimed in claim 115 is used.

135. (Currently Amended) ~~The use of a nanoparticle as claimed in any of claims 115 to 127~~
A method for at least one of enriching ~~and/or~~ and isolating specific CD4⁺-T-lymphocytes or CD8⁺-T-lymphocytes in vitro wherein a nanoparticle as claimed in claim 115 is used.

136. (Currently Amended) ~~The use of a nanoparticle as claimed in any of claims 115 to 127~~
A method for at least one of priming ~~and/or~~ and restimulating a CD4⁺- and/or CD8⁺-T-lymphocyte reaction in vitro wherein a nanoparticle as claimed in claim 115 is used.

137. (Currently Amended) ~~The use of a peptide vaccine as claimed in any of claims 128 to 131~~
A method for the active immunization of an animal or human organism against a protein

antigen wherein a peptide vaccine as claimed in claim 128 is used.

138. (New) The method of claim 55 which further comprises at least one of the following additional steps:

- a) preparing, by genetic engineering or chemical synthesis, suitable amounts of a peptide based on the determined amino acid sequence of the peptide fragment,
- b) preparing, by genetic engineering or chemical synthesis, suitable amounts of the first and second chains,
- c) preparing suitable amounts of peptide-presenting MHC molecules by joint incubation of the first chain, the second chain and the peptide prepared, and
- d) preparing a peptide vaccine in the form of a lyophilizate or an aqueous colloidal solution or suspension of the peptide-presenting MHC molecules.